METHOPRENE AS A PROTECTANT AGAINST FIVE SPECIES OF STORED-PRODUCT INSECTS IN WHEAT

by

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I DEDICATE THIS WORK TO MY WIFE MARIELOS, WHOSE UNTIRING DEVOTION HAS MADE IT POSSIBLE.

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INTRODUCTION

In recent years, the search for alternative treatments to malathion as a grain protectant has been intensive, due in part to the appearance of insect strains resistant to malathion. Some insect growth regulators (IGR) have been tested as grain protectants. Among those, methoprene has been one of the most successful and promising compounds against stored-product insects. Methoprene combines a low mammalian toxicity (a basic requisite for grain protectants) with a persistency greater than malathion. Also, methoprene is active against the more sensitive species at very low concentrations.

Methoprene has already proved an economically feasible and effective protectant for tobacco and peanuts, and has been labeled for these uses, among others.

Most documentation on methoprene as a grain protectant relates to laboratory-scale studies, and field-scale evaluations of methoprene are needed.

In this study, methoprene at two concentrations was evaluated as a candidate wheat protectant against five species of stored-product insects, and compared to malathion in an actual semifield-scale storage test.

The Juvenile Hormone.

The juvenile hormone (JH), secreted by the corpora allata, is essential for growth and development of young larval instars. Secretion of the endogenous hormone is interrupted by internal physiological mechanisms in the last instar larva. This results in replacement of the larval type of growth with development, by differentiation, of the pupal (in Endopterygote insects) or adult (in Exopterygote insects) structure. In general, embryonic development and metamorphosis are characterized by an absence of JH and by intensive differentiation (SLAMA, 1971).

The first extracts with JH activity were obtained by WILLIAMS (1956) from "adult males of the cecropia worm (Platysamia cecropia (L.))". In 1959, JH active substances were found in excrement of Tenebrio beetles and were identified as a sesquiterpenic alcohol (farnesol), and its aldehyde (farnesal). Both compounds were JH-active on Tenebrio and Rhodnius whereas they exhibited little or no activity on other species (SLAMA, 1971). Since that time, a series of related sesquiterpenoid juvenile hormone analogues (JHA), which have specific effects on various insect species, have been described. In 1971, SLAMA estimated the number of known JHA's to be greater than 500.

Insect Growth Regulators.

Insect growth regulators (IGR) include compounds that may affect moulting and metamorphosis by mimicking JH activity or by interferring with cuticle formation (NICKLE, 1979).

Embryonic development and metamorphosis are the insect stages most sensitive to JHA's, although in some species reproduction processes are sensitive also. METWALLY et al. (1972) reported that treating Trogoderma granarium (Everts) females with a JHA produced severe disorders of the ovaries which resulted in low egg hatchability. On the other hand, young larval instars and developing adult insects, which have high endogenous hormone titers, generally appear to be insensitive to JHA's. This was documented in the report by STRONG and DIEKMAN(1973), in which none of the 15 IGR's tested affected the life span of reproductively mature parent adults of 12 species of stored-product insects. However, AMOS and WILLIAMS (1977) reported that adult mortality in Sitophilus oryzae (L.), S. granarius (L.) and Rhyzopertha dominica (F.) was markedly increased when exposure to two JHA's (methoprene and hydroprene) occurred under unventilated conditions.

The critical period for JHA action on embryogenesis is believed to occur very soon after the eggs are deposited (SLAMA, 1971). MIAN and MULLA (1982b) found that methoprene caused almost complete egg mortality in Oryzaephilus surinamensis (L.) and R dominica (F.) but was less effective against eggs of Tribolium castaneum (HERBST). SLAMA (1971) also reported that the egg stage of some species showed complete insensitivity toward particular JHA's. KRAMER amd MCGREGOR (1978), testing five pyridyl and phenyl ether analogues on wheat or ground wheat medium, found no ovicidal effect on three stored grain moth species. Similarly, BHATNAGAR-THOMAS (1973) testing MTDD[1] on

1/. Methyl 3,7,11-trimetyl-7,11-dichloro-2-dodecenoate.

S. granarius (L.) reported no appreciable effect on egg hatchability.

Fecundity can be altered also by JH mimics. NICKLE (1979) indicated that in a treated population of almond moth (Ephestia cautella (Walker)) fecundity was reduced by the increased occurrence of sterile matings (i. e., matings in which females failed to produce any viable eggs) and by reduced survival of offspring to adulthood.

An increase in the developmental period of a species, mainly due to lengthening of the larval stage, is a common effect of JHA treatments. Hydroprene at 20 ppm prolonged the larval stage of red flour beetle (Tribolium castaneum (Herbst)) larvae, and individuals died in the larval stages or as adultoids (WILLIAMS and AMOS, 1974). Because lengthening of the developmental period is so dramatic in certain species, WILLIAMS and AMOS (1974) suggested that JHA's may prove more useful as grain protectants than as direct control agents.

Studies on the migration of IGR's into grain kernels showed that two days after treatment most of the chemical had accumulated in the aleurone layer. Much less was found in the germ, and only trace amounts in either the endosperm or the seed coat (ROWLANDS, 1976). The uneven distribution was believed to influence insect control in accordance with feeding habits of the particular store-product insect. In practice, however, effective control appears to be related to the susceptibility of the specific pest (STRONG and DIEKMAN, 1973) and to the ambient conditions under which exposure to the IGR takes place (AMOS and WILLIAMS, 1977), rather than on the insect feeding habit.

JHA's have shown specificity against closely related species of insects. AMOS et al. (1974) reported that of nine compounds tested, two were effective against Tribolium castaneum while none inhibited development of T. confusum (du Val). ZETTLER (1979) reported that a synthetic JHA (Hoffman-LaRoche Ro 20-3600) had a greater synergistic effect than piperonyl butoxide on pyrethrins applied against malathion-susceptible and malathion-resistant strains of the T. castaneum. No data were found describing synergism with other IGR's.

Methoprene Activity on Selected Stored-Product Insects.

Methoprene (isopropyl (2E-4E)-11-methoxy-3,7,11-trimethyl-2, 4-dodecadienoate), the insect IGR tested in this study, has also been designated as altosid, ZR-0515 and ENT-70460 (STRONG and DIEKMAN, 1973). Reported effects of the compound on certain stored-product insects are summarized in the following paragraphs.

Lesser grain borer (Rhyzopertha dominica (F.)). STRONG and DIEKMAN (1973) tested 15 IGR's on several species of stored-product insects and reported that most compounds only delayed emergence of the F-1 generation. However, altosid (methoprene) at 5 ppm on wheat completely prevented adult emergence of the lesser grain borer (LGB) during 16 weeks of continuous exposure and was the most active compound against the beetle. In addition, MCGREGOR and KRAMER (1975), in a 6-week trial, observed that methoprene applied to corn and wheat at 2 and 4 ppm reduced the F-1 population. MIAN and MULLA (1982a) also reported effective protection of wheat against LGB for more than a year

when methoprene was applied at 1, 5, and 10 ppm. The protection lasted only two months when rates of 0.1 and 0.5 ppm were used.

Two studies (STRONG and DIEKMAN, 1973; MCGREGOR and KRAMER, 1975) indicated that reproductively mature parent adults were not affected by methoprene treatments. However, AMOS and WILLIAMS (1977) found that with no ventilation in the storage container there was a marked increase in parental mortality of LGB and other species at all methoprene concentrations tested (1, 5, 10 and 20 ppm). This finding suggested the existence of a vapor effect.

Red Flour Beetles (Tribolium castaneum (Herbst.) and Confused Flour Beetle (Tribolium confusum (Jacqueline du Val). At 1 ppm on a medium of whole wheat flour: brewers' yeast (95:5 parts), LOSCHIAVO (1975) found survival of red flour beetle (RFB) larvae unaffected while survival was reduced dramatically at higher concentrations (19 and 8% survival at 10 ppm of methoprene technical and emulsifiable formulations, respectively). Similar results were obtained for the confused flour beetle (CFB). Although larval survival of CFB declined as methoprene concentrations increased, larvae appeared to be less sensitive to 10 ppm than RFB larvae. LOSCHIAVO (1975) also reported emergence of a small percentage (3%) of normal live CFB adults from 5 ppm methoprene-treated medium. WILLIAMS and AMOS (1974), however, reported complete suppression of RFB progeny when reared in flour milled from wheat treated with methoprene at 5 or 20 ppm. In this case adults were not produced; the individuals died either in the larval stage or as adultoids. In another study LOSCHIAVO (1976) found that no normal flour beetle adults (RFB or CFB)

emerged from wheat flour diets containing 10 or 20 ppm methoprene. Additionally, larvae that failed to pupate in the treated food continued to molt. Larvae surviving 120 days or longer after hatching were larger than normal and had more sclerotized integuments than those of normal larvae. EDWARDS (1976) also noted RFB larvae in flour treated with JH-1[1] had not completed metamorphosis 10 weeks after hatching. LOSCHIAVO (1975) reported that larval developmental time of RFB and CFB increased as the dosage of methoprene increased.

Reports disagree on the effect of IGR's on oviposition of flour beetles. LOSCHIAVO (1976) indicated that RFB did not lay eggs in treated diets containing methoprene at 5, 10 or 20 ppm. Later, MIAN and MULLA (1982b), using wheat flour treated with 5 ppm methoprene, reported that oviposition by RFB during a 2-week exposure period was not significantly different from that of controls. They suggested that ovipositional responses were affected by the age of adults at the time of exposure.

The descriptions of pupal-adult intermediates given by LOSCHIAVO (1976) for methoprene-treated diets, and EDWARDS (1976) for flour treated with JH-1 were similar. The head and thorax appeared normal, but the abdomen retained a pupal-like appearance.

Cross-resistance to a JHA (JH-1) in a strain of the RFB resistant to many conventional insecticides was reported by DYTE (1972). However, AMOS et al. (1974) determined that a malathion-resistant strain of the RFB did not display cross-resistance to

1/. Cis/trans mixture of synthethic methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (69% pure).

various JHA's. Although cross-resistance to methoprene in insecticide-resistant strains of houseflies (CERF and GEORGHIOU, 1972 and 1974), and induction of resistance to methoprene in a strain of the northern mosquito (Culex pipiens pipiens L.) (BROWN and BROWN, 1974) has been reported, no information is available regarding the presence of resistance to methoprene in flour beetles or in other species of stored-product insects.

Rice weevil (Sitophilus oryzae (L.); granary weevil (Sitophilus granarius (L.); maize weevil (Sitophilus zeamais Motschulsky). The insensitivity of weevils to the known JH's was recognized early. In 1971, SLAMA said: "From our experience we know that insects like the beetles of the family Scolytidae or Curculionidae are completely resistant against the known JHA's". Other authors have reported a strong tolerance to methoprene by Sitophilus weevils but not a complete resistance. STRONG and DIEKMAN (1973) found that the response of the rice weevil (RW) and granary weevil (GW), (Sitophilus oryzae (L.) and S. granarius (L.), respectively), to altosid (methoprene) at 50 ppm was a 46 and 92% reduction of F-1 adults, respectively. MCGREGOR and KRAMER (1975) applied methoprene at 10 ppm to wheat, and 13% reduction in the number of F-1 adults resulted. LOSCHIAVO (1976) reported a reduction of only 10% in F-1 progeny when RW parent adults were exposed to 10 ppm methoprene-treated wheat; a reduction of 61% was obtained at 20 ppm. In contrast, MIAN and MULLA (1982a) found that methoprene applied at 10 ppm to wheat gave 85% control of the F-1 progeny during an entire 12-month period. Although reports vary widely, a complete reduction in the F- 1 generation of Sitophilus species has not been achieved

by methoprene at doses up to 50 ppm. Even though it is likely that high methoprene concentrations can give effective protection to stored grain against weevils, the doses required are not economically feasible (MIAN and MULLA, 1982a).

AMOS and WILLIAMS (1977) demonstrated that Sitophilus species adults were sensitive to both methoprene and hydroprene when exposed under unventilated conditions. Different responses to ventilated vs unventilated conditions suggested a vapor effect. Sensitivity was shown not only in a marked increase in parental mortality but also in suppression of productivity. The authors hypothesized that methoprene and hydroprene may be extremely effective commodity protectants in bulk storages, but no data on large scale storage has been found to test their hypothesis.

Sawtoothed grain beetle (Oryzaephilus surinamensis (L.)); merchant grain beetle (Oryzaephilus mercator (Fauv.); flat grain beetle (Cryptolestes pusillus (Schonherr). Methoprene at 5 ppm apparently induced oviposition in the sawtoothed grain beetle (STGB) but also showed significant ovicidal activity (96% mortality). Most of the larvae which hatched from the surviving eggs died as first instars 1 or 2 days after hatching (MIAN and MULLA, 1982b). LOSCHIAVO (1975) exposed newly-hatched STGB and merchant grain beetle (MGB) larvae to various diets treated with methoprene at 1, 5 or 10 ppm. Larvae fed treated corn meal failed to pupate; in wheat flour: brewers' yeast (95:5 parts) pupation occurred, but no live adults had emerged after 45 days; larvae in treated rolled oats did not survive. Methoprene was the most active compound among the 15 IGR's tested against the

STGB by STRONG and DIEKMAN (1973); in oatmeal treated with methoprene at 5 ppm, a 99% reduction in the number of larvae was obtained; larvae died before pupating.

MCGREGOR and KRAMER (1975) reported that a 3-week exposure of STGB adults to methoprene-treated wheat or corn (2 to 10 ppm) was not toxic to adults, and eggs subsequently deposited on untreated grain by the exposed females developed normally. This suggested that methoprene does not affect the ability of temporarily-exposed adults to produce viable eggs after they are removed from a treated medium. STRONG and DIEKMAN (1973) also reported that parent adults were not affected by methoprene treatments.

Although several investigators have studied the effect of methoprene on the STGB and one tested activity of a JHA on MGB, there were no reported tests on flat grain beetles (FGB).

Indian meal moth (Plodia interpunctella (Hubner). Reports generally agree that methoprene effectively controls the F-1 population of the Indian meal moth (IMM). STRONG and DIEKMAN (1973) reported 100% reduction of the F-1 population at doses of 5, 10 or 50 ppm on a moth rearing medium. LOSCHIAVO (1975) also found good control of F-1 adults during a 5-month period when methoprene was applied to whole wheat flour: brewers' yeast (95:5 parts) at 5 and 10 ppm. In his experiment 100% control was achieved when an EC (52.7% AI) formulation of methoprene was used at 5 ppm, but when a technical formulation (62.1% AI) was used at the same concentration, some adults (2% of the number in an untreated medium) emerged. MCGREGOR and KRAMER (1975), however, found that technical and emulsifiable formulations of

methoprene gave similar results.

Larvae of IMM are not killed soon after exposure to a methoprene-treated medium and webbing does occur in treated media. STRONG and DIEKMAN (1973) reported webbing activity decreased to about half that observed in the control treatments in moth medium treated with 50 ppm. Although MCGREGOR and KRAMER (1975) reported a significant decrease in webbing activity of IMM larvae reared on treated grain, they gave no further details.

Wheat.

Newly-harvested 1982-crop wheat (combine-clean), which had no chemical treatment after harvest, was purchased from the Kansas State University Agronomy Farm. The wheat was an experimental Newton-type hard red winter variety, KS-75210. Approximately 700 bu were placed in a metal bin next to the experimental site 20 days before the experiment started. The grain was sampled as it entered the auger conveying grain into the bin. A composite sample, analyzed by the Kansas State Grain Inspection Department, was graded as follows:

U.S. No.4 Hard Red Winter Wheat	Dockage: 0.5%
Weight per bushel: 55.5 lbs	Total Damage: 0.9%
Moisture: 12.3%	S & BK: 1.4%
Foreign Material: 0.1%	Defects: 2.4%

The limiting factor for grade designation was test weight. All other factors were within the limit for U.S. No.1, Hard Red Winter Wheat.

Bins.

Twelve corrugated metal bins of 50-bu capacity each were used in this experiment. Bins had flat bottoms and removable lids which could be fastened in place. Bins were placed in a pole building in rows of five, two and five running from south to north. The roof of the building protected the bins from rain and sun, but the absence of solid walls permitted free movement of air currents. Each bin was placed on a square plywood base

standing on a layer of sand about 5 cm deep. Bins were filled within 5 to 10 cm of the top with 3,000 lbs of treated wheat. After the bins were filled, the surfaces were leveled and bins were covered with conical-shaped lids (approx. 17.5 cm high at the center). Bin lids were removed only to introduce insects and to collect samples.

Insects.

The following species of stored-product insects were used: Indian meal moth (Plodia interpunctella (Hubner)); flat grain beetle, (Cryptolestes pusillus (Schoenherr)); sawtoothed grain beetle, (Oryzaephilus surinamensis (L.)); red flour beetle, (Tribolium castaneum (Herbst)); and lesser grain borer (Rhyzopertha dominica (F.)). All insect cultures used to infest bins or to start bioassays were obtained from the KSU Department of Entomology's Stored-Product Insects Laboratory rearing room. No information was available on resistance or susceptibility of colonies to insecticides.

The media used to rear insects were: hard red winter wheat, for lesser grain borer (LGB); rolled oats: active yeast mixture (95:5 parts), for flat grain beetle (FGB) and sawtoothed grain beetle (STGB); whole wheat flour: active dry yeast (95:5 parts), for red flour beetle (RFB); and a mixture of 80 parts of chick mash, 6.66 parts of honey (warm), 6.66 parts of glycerol and 6.66 parts of water (hot), and one teaspoon of brewers' yeast for each quart jar of medium, for Indian meal moth (IMM).

Prior to infestation of bins, adult beetles were collected from cultures in groups of 200 and kept in small screened jars

until their release. Moth eggs were collected from bottom-screened oviposition jars. When released, LGB and FGB were <28 days old; STGB and RFB, <7 days; and IMM eggs, <2 days.

Protectants.

The grain protectants used in this experiment were methoprene (an insect growth regulator) and malathion (a common grain protectant currently in use, worldwide). The formulation of methoprene contained 600 g A. I. per liter, and was an emulsifiable concentrate called Diacon^{R} (Zoecon Co., Palo Alto, Calif.). The formulation of malathion used was a commercial emulsifiable concentrate, containing 600 g AI per liter.

Concentrates were diluted to provide the following treatments, when applied at a rate of 1.88 ± 0.1 liters per 3,000 lb (approx, 37.6 ml/bu): methoprene, 10 and 20 ppm; malathion, 10 ppm (Appendix 1). Dilutions of the two grain protectants were prepared just before the treatments were applied. For the control treatments, grain was treated with water at the same rate. The malathion treatment was equivalent to that recommended on the label of the commercial product used, i. e., 1 pint 57% E.C. premium grade malathion per 1,000 bu.

Application of Protectants to Grain.

Wheat was augered from the holding bin in 3,000-lb lots into a tote bin on a truck bed. Grain was treated as it entered a 20-ft auger which mixed grain and treatment before discharging wheat into 50-bu bins. The sprayer equipment delivered an average of 132.95 ml/min when pressure on the sprayer tank was preset at 8 psi. A 15-lb CO_2 tank (ca. 1,000 psi) served as the source for

constant pressure on the 5-gal stainless steel beverage-type sprayer tank. The flat nozzle (No. 650067, Spraying Systems Inc., Wheaton, Ill.) was directed onto the grain stream exactly at the point where grain entered the 20-foot auger (Appendix 2).

The control (water) treatment was applied first, followed by 10-ppm methoprene, 20-ppm methoprene and 10-ppm malathion. Each treatment was replicated three times. Before the malathion treatments, all wheat was removed from the auger, and several bushels of wheat run through the auger to remove methoprene residues. Grain was treated on two consecutive days with six bins treated each day.

Bins were filled within 5 to 10 cm of the top with 3,000 lbs of treated wheat. After the bins were filled, the surfaces were leveled and bins were covered with non-airtight solid lids. Bin lids were removed only to introduce insects and to collect samples.

Infestation of Bins.

Six days after treatments were applied, wheat in bins was infested at three locations: on the surface, at the bottom and at mid-depth near the south side. At each location, groups of 200 adult beetles of each species were introduced, one species at a time, so that each bin received 2,400 individuals. Thus, total initial level of infestation (excluding IMM) was 0.8 insects per pound or 1.76 insects per kilogram. The initial level of infestation of any beetle species was 0.2 insects per pound or 0.44 insects per kilogram. Insects released on the surface were sprinkled on the wheat near the center. The other groups were

introduced with a hollow probe of the type used to apply solid fumigants. The probe was inserted into the bulk, the insects were dropped into it and then the probe was pulled out leaving the insects behind. Fifty (50) IMM eggs were spread over the surface of wheat in each bin. Bins were infested on two successive days. The first day, bins were infested with LGB and RFB; the following day STGB and FGB and IMM eggs were added.

Sampling of Bins.

Initial sampling. Wheat designated for each of the 12 bins was sampled immediately before treatment. To form a composite sample, handfuls were taken periodically from the grain stream as wheat was unloaded from the holding bin into the scale.[1] Test weight and moisture content were determined on the composite sample. The test weight was measured three times on each sample, and the average reported. Bins were sampled three days after treatment by drawing nine small probe samples from each bin to form a composite sample (details given in "monthly sampling" section). After-treatment moisture contents were determined and 500-g portions separated with a mechanical grain divider for analysis of grain protectant residues. Samples from bins containing methoprene-treated grain were sent in metal containers to the Zoecon Corporation, Palo Alto, California. Malathion-treated grain was frozen at -20 C until residue analyses were made at the U.S. Grain Marketing Research Laboratory, Manhattan, Kansas.

1/ A 1,100-lb capacity Fairbanks scale with a built-in Honeyville hopper from Metalworks Inc., Topeka, Kansas.

Monthly sampling. Samples for determination of moisture content, protectant residues, and progeny emergence were withdrawn from each bin at 30-day intervals for 5 months. On each of the five sampling dates, nine small-probe samples (approximately 1,000 g) were drawn from each bin. The probe was 63" long, 7/8" outside diameter, double-tube, brass, chrome-plated, and compartmented by using rubber stoppers.

A template placed on top of the bin was used to identify the nine probe sites (Appendix 3). This metal guide divided the surface area of the bin into nine sections, a small center section and eight equal-area sub-sections which were further divided into four sections each of equal area to the center section. The center point was always probed; and within each sub-section the probe site was selected by lot from the four positions available.

Each of the nine samples was divided into equal upper and bottom portions. Each portion was sieved (U.S. Std. No. 10) and thrus collected in plastic bags. Then all the overs of all probed grain from a bin were composited. These samples were mechanically divided into portions to analyze for moisture content (30 g), protectant residues (500 g), and progeny emergence (250 g). Monthly samples from malathion treated bins were kept frozen at -20 C until tested for residues; monthly samples from methoprene-treated bins were sent in sealed quart metal cans, to Zoecon Co., Palo Alto, California.

Analyses.

Moisture content. The method of HART et al. (1959) for whole seed wheat was used (10 g dried at 130 C for 19 hrs). Each determination was made in triplicate and reported as an average.

Insect counts. Thrus from sieved probe samples were examined for adults and larvae insects. Counting and identification of adults was done mainly by the naked eye. An illuminated magnifying lens was used for larval and pupal counting. A microscope was used when a question arose in identifying small larvae in mixed populations.

Progeny emergence. Progeny emergence was determined by placing 250-g portions from each bin in wide-mouth quart Mason jars. Jars were covered with a screened lid over filter paper and were placed on shelves in a rearing room at 27±1C and 65±5% r.h. Counts of emerged progeny were made after 50 days.

Malathion residues. Protectant residues in malathion-treated bins were assayed at 3, 30, 90, 120 and 150 days after treatment. The analytical procedure used was the standard procedure used at the U.S. Grain Marketing Research Laboratory (Appendix 4). Extraction efficiency for this procedure was not determined on wheat analysed during this experiment. Thus, residues reported were not corrected for extraction efficiency.

Methoprene residues. Methoprene residues were assayed at 3, 30, 90 and 150 days after treatment. Only samples from bins 1 (control), 5 (10-ppm methoprene), and 9 (20-ppm methoprene) were assessed.

Actual analyses of samples were made by an independent laboratory using Zoecon Method No. 141-0679-ORM, "Residue

determination of methoprene (Altosid^{R} Insect Growth Regulator) in animal fat and in stored products (peanuts and whole grain kernels)".

Bioassays.

Insect species used in the multiple-species infestation of the bins were bioassayed individually on wheat removed from the experimental bins to assess (1) survival and development of progeny of adults exposed to the treated grain for a short period and (2) ability of the species to develop a population on treated grain. Bioassays were begun 7, 45 and 90 days after treatments were applied.

Wheat samples for assays. Four large-probe (a ten openings, 63" long 1 3/8" outside diameter, double-tube, brass, compartmented probe) samples were obtained from each bin at each cardinal point about 20 cm from the bin wall. Each probed sample was sieved and insects were removed. Then, the four samples were composited (thrus replaced after insects were counted) and placed in plastic bags which were sealed. The composited samples were frozen for not less than 3 days in order to kill any insect forms present.

Samples were removed from the freezer and allowed to warm to room temperature. Each was tumble-mixed in the plastic bag for at least 1 minute and divided into 15 50-g portions and 5 100-g portions. The 50-g portions were each placed in a 4-ounce (baby food) jar and the 100-g portions in wide-mouth quart Mason jars. All jars had screened (80 mesh) lids, and the quart jar lids were additionally fitted with filter paper.

Survival-and-development-of-progeny. Three 50-g portions from each bin were each separately infested with 25 adults of a given beetle species or with 25 eggs of IMM. Jars were placed in the rearing room. A Walton Humidifier unit was used to maintain 65± relative humidity, and an electric heating unit equipped with a fan kept the temperature at 27±. The room was kept in the dark except for the short periods of time when jars were taken out for analysis.

Survival was evaluated after a 7-day exposure period. At that time adult beetles were removed from jars, the live and dead recorded. To minimize disturbance of the grain during removal of adults, the contents of a jar was spread over a paper towel; live adults were captured with an aspirator, and dead ones were picked up with forceps. The sample was carefully poured back into the original jar, and returned promptly to the rearing room.

Insect progeny was determined 50 days after initiation of the assay. The analysis included determination of numbers of live and dead adults, pupae and larvae. The percentage of degermed kernels was determined for wheat samples infested with IMM. For this, 100 kernels, taken randomly from the jar, were examined under a magnifying lens, and the number of partially or completely degermed kernels was recorded.

As an additional analysis, all wheat samples infested with LGB were X-rayed at 80 days to check for internal infestation. Samples were sieved prior to radiographing to separate the wheat from any other contaminants (insects, dust, etc.). After X-raying, samples were returned to the original jars with the

contaminants removed initially.

Assessment of population development. Population development was assessed on 100-g portions of treated grain, each infested with 50 adults of one of the beetle species or 50 eggs (IMM). The jars were left undisturbed in the rearing room for 50 days. Analyses performed at the end of the 50-day period on and dates were exactly the same as indicated above for the small jars. All jars were returned to the rearing room after analysis and kept for 50 more days. Population development was assessed at the end of the 100-day period.

Grain Moisture Content.

Mean grain moisture in control treatments was 0.3% above the mean moisture level in 10-ppm methoprene treatment, and 0.6% above 20-ppm methoprene and 10-ppm malathion treatments (Table 1). These differences were present throughout most of the experiment, regardless of the changes which occurred in the grain moisture as a response to the ambient air conditions. Also, grain at the end of the experiment, when compared with grain at the beginning of the test, exhibited an increase in moisture content that ranges from 0.2 to 0.6%. These increases in grain moisture content were most likely caused by seasonal changes in ambient conditions, rather than the development of insect populations, or any other factor.

During the first month of storage, moisture increased slightly in all bins (approx. 0.1%). During the second month an equal increase of 0.4% occurred in all bins. Moisture content dropped 0.2% during the third month of storage, and then stabilized during the cold months of November, December and part of January. The increases observed during the first two months could be explained by the high ambient relative humidity which predominated during that time (Appendix 5). Thereafter, grain moisture contents stabilized despite the low ambient relative humidity. Calculated grain and air water vapor pressures (CHUNG-PFOST, 1967) indicate small pressure differentials favor drying of grain during the cold months. Small vapor pressure differentials and cold temperatures must have slowed any vapor

Table 1. Monthly average moisture content (%, wet basis) of infested, treated wheat in 50-bu bins during 5-months storage.[a]

TREATMENT	MONTHS AFTER TREATMENT						MEAN MOISTURE CONTENT
	0 (AUG)	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
CONTROL	13.3	13.3	13.7	13.5	13.5	13.5	13.5
METHOPRENE 10 ppm	12.8	12.9	13.3	13.1	13.1	13.4	13.2
METHOPRENE 20 ppm	12.6	12.7	13.1	12.9	12.9	12.9	12.9
MALATHION 10 ppm	12.6	12.7	13.1	12.9	12.8	12.9	12.9

[a]Average of three replications.

transfer process. ABBOUDA (1984) also found that moisture change was minimal during winter in grain stored in Kansas.

Grain Temperature.

Grain temperature changes observed during the experiment were not associated consistently with any of the treatments, nor did any of the bins show evidence of heating caused by insect activity or by any other source (Table 2).

At the end of the second month, temperatures in bins began to decline and by the end of the third month were no longer suitable for sustaining active insect populations (HALL, 1970). Average grain temperatures during the last 3 months of storage presumably were low enough to stop insect activities including reproduction, and during the last 2 months, to kill non cold-hardy species (COTTON and WILBUR, 1974). Regardless of the low temperatures, live adults and live larvae of the IMM, STGB and FGB species were recovered during the last two monthly samplings.

Temperatures at different depths in bins are not reported here because little variation was observed from the average reported in Table 2.

Protectant Residues.

Protectant residues (methoprene or malathion) found on grain three days after treatment averaged 60.8, 68.3 and 71.4% of the calculated applied dosages for 10-ppm and 20-ppm methoprene, and 10-ppm malathion treatments, respectively (Table 3). These data indicate a protectant loss, by the third day, of 39.2, 31.7 and 28.6% for the three treatments. DESMARCHELIER and BENGSTON (1978) indicated that an average of 15% of a protectant may be lost by

Table 2. Average temperatures (C) in infested, treated wheat in 50-bu bins during 5-months storage.[a]

TREATMENT	MONTHS AFTER TREATMENT				
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)
CONTROL	20.9	16.3	9.2	1.7	2.3
METHOPRENE 10 ppm	22.3	16.0	9.1	1.8	1.8
METHOPRENE 20 ppm	21.5	15.7	8.9	2.0	2.1
MALATHION 10 ppm	21.7	16.2	8.8	2.7	1.9

[a]Average of three replications, each consisting of four readings at different depths in the center of the bin.

vapor losses, missing of target, and other processes at time of application. The losses three days after treatment for this experiment were nearly twice as much. LaHUE and DICKE (1977) in a trial with several grain protectants reported recoveries at 24 hr after treatment ranging from 95.2 to 74.7%; for malathion the recovery was 82.7%. It is likely that drifting of some of the fine spray generated by the pressurized spraying equipment used, played an important role in protectant losses in this experiment. Wheat temperatures at time of treatment (23.8 to 28.2 C), also may have enhanced vapor losses and fast initial breakdown.

However, residue recoveries in this experiment do not seem low if compared with other trials in which large lots of grain are treated and problems of a practical nature are faced. One such trial by TYLER and GREEN (1968) reported residue recoveries ranging from 50.0 to 81.7% from samples collected inmediately after treatment.

Part of the residue losses may be attributed to sample handling procedures. All samples contacted several surfaces prior to their final analysis, including probe, trough, plastic bags, sieves, divider, and metal cans. This handling may have been responsible for losses of protectant present on the surface of kernels. However, the rapid penetration of IGR's into kernels as demonstrated by ROWLANDS (1976), minimizes the loss of protectant when freshly treated grain is handled.

Malathion residues degraded faster than methoprene residues. One month after treatments were applied, malathion residues in samples accounted for only 47.1% of the residues present at three days, while 88.7% and 79.7% of methoprene residues were still

Table 3. Protectant residues (ppm) recovered from treated wheat at different intervals, during 5-months storage.[a]

TREATMENT	CALCULATED DOSE APPLIED	DAYS AFTER TREATMENT				
		3 (AUG)	30 (SEP)	90 (NOV)	120 (DEC)	150 (JAN)
CONTROL[a]	0.0	0.0	0.0	0.0	0.0	0.0
METHOPRENE 10 ppm[b]	10.2	6.2	5.5	4.5	-[c]	5.2
METHOPRENE 20 ppm[d]	20.2	13.8	11.0	9.1	-[c]	10.7
MALATHION 10 ppm[e]	9.8	7.0	3.3	2.6	2.7	2.3

[a]All control bins were checked for malathion residues. Only bin 1 was checked for methoprene residues.
[b]Only samples from bin 5 were examined for methoprene residues.
[c]Data not available.
[d]Only samples from bin 9 were examined for methoprene residues.
[e]Each residue is an average of three replications.

present after the same period in the 10- and 20-ppm treatments, respectively. Due to the low ambient temperatures which cooled the grain during the fourth and fifth months of the experiment (November and December), degradation of protectants was less. Malathion showed a slight decrease from 3.3 to 2.3 ppm, and methoprene in 10- and 20- ppm treatments decreased from 5.5 and 11.0 ppm to 5.2 and 10.7 ppm, respectively. Low grain temperatures favoring stability of malathion applied to wheat has also been reported by WATTERS and MENSAH (1979).

Methoprene was more stable than malathion under the same low temperature conditions. At the end of the five-month storage period, only 32.8% of the malathion residue found at three days was still present, while 83.8 and 77.5% of the 3-day methoprene residue were found in the 10- and 20-ppm treatments, respectively. Reports on persistency of methoprene are scarce. ROWLANDS (1976) indicated that methoprene (Altosid) residual half-life at 20 C in freshly-harvested wheat of 19% moisture was 2-3 weeks. In contrast, MIAN and MULLA (1982a) reported that, apparently, methoprene persists in wheat grain (13.5% moisture) for up to 12 months or longer, depending upon the moisture content of grain and storage temperature. Their assessment was based on the residual activity of methoprene in treated grain against some pests of stored products, and not on analytical assays to quantify its presence.

Insect Infestation.

Six days after treatments were applied, all bins were infested with five stored-product insect species. Development of

insect populations were thereafter monitored by monthly samplings. Average total number of insects recovered from samples, or insects found on the surface of bins (in the case of IMM) are reported in Tables 4 through 10. Surface infestations by IMM, and bulk infestations by beetle species, are discussed separately.

IMM infestation The grand total (GT) number of all IMM forms (larvae, pupae, adults; live or dead) found in control bins was significantly higher than that found in malathion or either of the two methoprene treatments (Table 4). This indicated that all three insecticide treatments effectively reduced the number of IMM. Additionally, methoprene at the two levels tested (10 and 20 ppm) permitted the development of a significantly smaller GT number of all IMM forms than the 10-ppm malathion treatment. On only one occasion (after three months) was the number of all IMM forms found in malathion-treated bins significantly higher than the numbers from either of the methoprene treatments. Data on malathion-treated bins at three months also indicated that malathion was losing its effectiveness in controlling IMM insects, since a population build up obviously was underway. No significant differences were observed between the two levels of methoprene .

At all five sampling dates the numbers of all IMM forms found in the control bins were significantly higher than the numbers recovered from any of the insecticide-treated bins.

In general, the number of IMM adults in monthly samples (Table 4) indicated a population development tendency similar to that of all IMM forms, i.e., a rapid increase in population

Table 4. Average total numbers of live and dead Indian meal moths from the surface of 3,000-lb lots of wheat at monthly intervals, during 5-months storage.[ab]

TREATMENT	MONTHS AFTER TREATMENT 1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	GRAND TOTAL[c]
			ALL FORMS			
CONTROL	7.0 a	189.0 a	93.7 a	25.0 a	20.0 a	334.7 a
METHOPRENE 10 ppm	1.0 b	6.0 b	0.3 c	2.0 b	1.0 b	10.3 c
METHOPRENE 20 ppm	0.0 b	1.7 b	0.7 c	3.0 b	1.7 b	7.0 c
MALATHION 10 ppm	0.7 b	14.3 b	26.0 b	8.3 b	4.7 b	54.0 b
			ADULTS			
CONTROL	0.0 a	169.7 a	84.3 a	9.3 a	8.7 a	274.3 a
METHOPRENE 10 ppm	0.3 a	2.3 b	0.0 b	0.7 b	0.7 b	3.3 b
METHOPRENE 20 ppm	0.0 a	1.0 b	0.0 b	1.7 b	0.7 b	3.7 b
MALATHION 10 ppm	0.0 a	6.7 b	11.7 b	2.7 b	3.7 b	22.0 b
			LARVAE			
CONTROL	6.3 a	19.3 a	9.3 b	15.7 a	8.7 a	59.3 a
METHOPRENE 10 ppm	0.7 b	3.7 b	0.3 c	1.3 b	0.7 b	6.7 c
METHOPRENE 20 ppm	0.0 b	0.7 b	0.7 c	1.3 b	0.7 b	3.3 c
MALATHION 10 ppm	0.7 b	7.0 b	14.3 a	5.7 b	3.7 b	31.3 b

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Grand total is the sum of the five monthly average total number of IMM forms reported for each treatment.

during the first three months, followed by a sharp decrease due to low temperatures. The only outstanding difference found when using the variable 'number of adults' to compare the treatments statistically, was that there were no signigicant differences in the number of adults between malathion and either of the two methoprene treatments. This occurred even though the GT adult infestation was six-fold greater in malathion-treated bins than in methoprene bins.

Recovery of adults was greatest at the end of the second month. A second generation had already emerged from control bins, and the population evidently was increasing. The number of adults emerged in insecticide-treated lots of grain was statistically smaller than that of the control lots.

In general, similar results were obtained when the number of IMM larvae found in bins was analyzed (Table 4). However, at three months the number of larvae found in malathion bins was even significantly higher than the number of larvae found in control bins. This provided further evidence that malathion had lost its effectiveness in controlling IMM. Production of larvae in the malathion treatment was delayed when compared to the control treatment.

Infestation by Beetle Species.

Total infestation. Total numbers of all stages (live or dead) of all four beetle species (LGB,RFB,STGB, and FGB) used this experiment are reported in Table 5. Control bins had a total insect infestation significantly greater than that found in the other three treatments. Two to three times more insects were

recovered from the control bins, indicating that both malathion and methoprene treatments significantly reduced the total infestation. There were no statistical differences between 10- and 20-ppm methoprene treatments, nor between the two levels of methoprene and the 10- ppm malathion treatment. All three protectant treatments were statistically equivalent in protecting wheat from beetle species during the five-month storage period.

Monthly recoveries in control bins were always higher than from other treatments, but not always statistically different. Significant differences were more easily detected when comparing accumulated totals for the five-month storage period, than when comparing data from a single monthly sampling. This may be an important consideration when monitoring populations: isolated samplings may fail to reveal the significance of a population buildup, while accumulated totals may be more effective. BARAK and HAREIN (1982) indicated that conventional probe sampling as opposed to trap sampling, may underestimate insect populations. The use of accumulated totals may help in narrowing this gap when conventional probe sampling is still the choice.

Precision of probe sampling can be further improved if the best variable is chosen for comparison. Table 5 shows that the use of the number of adults as the response variable failed in all cases but one (two months after treatment) in finding differences between treatments. Best separation of protectant treatments was obtained when using the number of larvae (Table 5) as the response variable, both for the monthly estimates of infestation and for the total infestation. This was particularly true for the malathion treatment.

Table 5. Average total combined numbers of live and dead lesser grain borer, red flour beetle, sawtoothed grain beetle and flat grain beetle forms (per 1,000 g) from 3,000-lb lots of wheat at monthly intervals after treatment, during 5-months storage.[ab]

TREATMENT	MONTHS AFTER TREATMENT					TOTAL INSECT INFEST.[c]
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
			ALL FORMS; ALL SPECIES			
CONTROL	8.3 a	18.1 a	14.0 a	9.7 a	19.5 a	69.5 a
METHOPRENE 10 ppm	2.1 a	7.6 ab	6.4 ab	6.6 a	6.1 b	28.8 b
METHOPRENE 20 ppm	3.1 a	6.9 ab	2.2 b	3.9 a	3.6 b	19.8 b
MALATHION 10 ppm	4.7 a	1.4 b	6.1 ab	3.9 a	2.6 b	18.7 b
			ADULTS; ALL SPECIES			
CONTROL	5.1 a	15.5 a	8.6 a	4.8 a	10.3 a	44.3 a
METHOPRENE 10 ppm	1.4 a	3.3 ab	4.3 a	2.8 a	3.6 a	15.4 a
METHOPRENE 20 ppm	3.1 a	4.0 ab	2.2 a	1.4 a	1.5 a	12.2 a
MALATHION 10 ppm	4.6 a	1.4 b	6.1 a	3.9 a	2.6 a	18.7 a
			LARVAE; RFB, STGB, FGB			
CONTROL	3.2 a	2.5 ab	5.4 a	4.9 a	9.3 a	25.3 a
METHOPRENE 10 ppm	0.7 b	4.3 a	2.1 b	3.8 ab	2.5 ab	13.4 b
METHOPRENE 20 ppm	0.0 b	2.9 ab	0.0 b	2.5 ab	2.2 ab	7.6 bc
MALATHION 10 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Total insect infestation is the sum of the five monthly average total numbers of LGB, RFB, STGB and FGB insects recorded for each treatment.

Lesser grain borer. Only adults were considered in the analysis for lesser grain borer (LGB).

There were no statistical differences between grand totals (GT) of LGB adults for the different treatments (Table 6). Grand totals from methoprene treatments were the lowest (1.4 and 1.1 adults per 5 kg, for 10- and 20-ppm treatments, respectively). At the same time, they were less than expected based on densities originally placed in the bins (2.2 adults per 5 kg). The number of LGB adults recovered from the control bins was equal to that expected for no population increase. This could mean that there was no increase in untreated grain. However, 250-g samples collected monthly and incubated to estimate internal infestation (Table 7) showed that wheat samples had been invaded by LGB immature larvae by the time of the first sampling. Therefore, a low recovery of LGB insects in monthly samplings probably was due to the sampling technique, which probably underestimated the LGB population (BARAK and HAREIN, 1982); or to other unknown factors which limited the development of LGB under the bin conditions. The possibility of cross-contamination of control wheat with either of the protectants was not likely because of the order in which lots of wheat were treated.

Malathion treatment showed the largest recovery of LGB adults, a four-fold increase over the expected value under no population increase, but was not statistically different from the others. All were dead, supposedly killed soon after being placed in the bins containing wheat treated with malathion. Most adults recovered from malathion-treated bins were drawn at the third monthly sampling. It may seem that malathion at that time

Table 6. Average total numbers of lesser grain borer adults (per 1,000 g) from 3,000-lb lots of wheat at monthly intervals after treatment, during 5-months storage.[abc]

TREATMENT	MONTHS AFTER TREATMENT					GRAND TOTAL[d]
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
CONTROL	1.1 a	0.7 a	0.0 b	0.4 a	0.0 b	2.2 a
METHOPRENE 10 ppm	1.0 a	0.0 a	0.3 b	0.0 a	0.0 b	1.4 a
METHOPRENE 20 ppm	0.0 a	0.7 a	0.4 b	0.0 a	0.0 b	1.1 a
MALATHION 10 ppm[e]	1.8 a	0.0 a	5.1 a	0.7 a	0.7 a	8.3 a

[a]Average of three replications.
[b]Includes all live and dead adults.
[c]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[d]Grand total (GT) is the sum of the five monthly average total number of LGB adults recorded for each treatment.
[e]Only dead adults were recovered from malathion-treated wheat.

had lost its effectiveness in controlling LGB, and that an increase in population was underway. However, it is more likely that the 5.1 insects/kg recovered at three months after treatment was the result of a clumped distribution in the grain bulk. This type of distribution might have occurred because of the manner in which insects were introduced into the treated wheat and by the fast killing action of malathion. A rapid and high mortality of LGB adults is achieved when exposure to malathion occurs under conditions similar to those used in this experiment. HYARI, et al (1977) reported 95.2% mortality of LGB adults when exposed to wheat containing just 2.9 ppm malathion; LaHUE and DICKE (1977) reported 93.9% mortality in LGB adults exposed to wheat containing 3.8 ppm malathion. Fast killing action has also been reported by LaHUE and KADOUM (1979), who obtained 73.8 and 100% mortality after 6 and 24 hr, respectively, when LGB adults were exposed to plywood surfaces treated with malathion sprays.

The number of LGB adults recovered monthly were, in general, low, with the exception of the recovery made in the malathion-treated bins at three months. Recoveries of LGB adults from malathion treatments were higher than the other treatments on two occasions: at three and five months after treatment. Methoprene treatments showed the lowest infestation by LGB throughout monthly samplings.

Red flour beetle. Table 8 indicates the numbers of all RFB forms (including larvae, pupae and adults; live or dead) found in samples drawn from 3,000 lb wheat lots during the five-month storage period. Grand totals (GT) for all forms and adults show that there were no significant differences between treatments.

Table 7. Lesser grain borer and flat grain beetle adults from monthly wheat samples (250 g) that were sieved and reared for 50 days.[abcd]

TREATMENT	SPECIES									
	LGB					FGB				
	MONTHS AFTER TREATMENT									
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)
CONTROL	8.0 a	2.3 a	3.3 a	0.7 a	0.0 a	6.0 a	7.6 a	2.7 a	1.3 a	0.7 a
METHOPRENE 10 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.7 b	0.0 b	0.0 b	0.0 b
METHOPRENE 20 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
MALATHION 10 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b

[a]Average of three replications.
[b]All samples were sieved before rearing to remove external infestation.
[c]Besides adults reported in this table, other insects recovered were as follows: 1 live and 1 dead RFB larvae at 2 months in methoprene 10ppm sample; 2 dead RFB larvae, and 1 live, 1 dead IMM larvae at 3 months in methoprene 10ppm sample.
[d]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

The numbers of all insect forms recovered from methoprene-treated wheat were larger than those numbers from the control or malathion treatments. This was due mainly to the recovery of RFB larvae and not to the recovery of adults. Numbers of RFB larvae in methopene-treated wheat were equal to or greater than in the controls, but significantly greater than those in malathion-treated wheat, in which no larvae were recovered. Evidently, viable eggs were laid by the RFB adults in methoprene-treated wheat as suggested by the several larvae recovered. This is not in agreement with LOSCHIAVO (1976), who indicated that no eggs were laid by the RFB in food treated with 5-ppm methoprene or more.

Although there were no significant differences between control and methoprene treatments in the recovery of RFB larvae during the experiment, numbers of larvae in methoprene treatments were consistently higher, both monthly and for the entire storage period. This can partially be explained by the inability of RFB larvae to molt in methoprene-treated wheat. This would increase the chance for new F-1 individuals to be collected while in the larval stage during successive monthly samplings. RFB larvae stayed in this stage longer than three months in bioassays. LOSCHIAVO (1976) reported that some RFB were still larvae after 150 days in a methoprene-treated medium.

The populations of RFB adults were not significantly different among treatments, although more than twice as many adults were recovered from malathion as from the other treatments during the storage period. All adults in malathion treatments might have been expected to die soon after being placed in or on

Table 8. Average total numbers of live and dead red flour beetle forms (per 1,000 g) from 3,000-lb lots of wheat at monthly intervals after treatment, during 5-months storage.[ab]

TREATMENT	MONTHS AFTER TREATMENT					GRAND TOTAL[c]
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
			ALL FORMS			
CONTROL	1.1 a	0.4 b	0.4 a	3.1 a	2.1 a	7.0 a
METHOPRENE 10 ppm	1.0 a	3.6 a	1.0 a	3.8 a	3.6 a	13.0 a
METHOPRENE 20 ppm	2.4 a	2.9 ab	0.4 a	2.5 a	2.2 a	10.4 a
MALATHION 10 ppm	1.1 a	0.0 b	0.7 a	2.5 a	1.5 a	5.7 a
			ADULTS			
CONTROL	0.7 ab	0.4 a	0.0 a	1.0 ab	0.0 a	2.1 a
METHOPRENE 10 ppm	0.3 b	0.0 a	0.0 a	0.4 ab	1.1 a	1.8 a
METHOPRENE 20 ppm	2.4 a	0.0 a	0.4 a	0.0 b	0.0 a	2.8 a
MALATHION 10 ppm	1.1 ab	0.0 a	0.7 a	2.5 a	1.5 a	5.7 a
			LARVAE			
CONTROL	0.4 a	0.0 a	0.4 a	2.1 a	2.1 a	4.9 ab
METHOPRENE 10 ppm	0.7 a	3.6 a	1.0 a	3.5 a	2.5 a	11.2 a
METHOPRENE 20 ppm	0.0 a	2.9 ab	0.0 a	2.5 a	2.2 a	7.6 a
MALATHION 10 ppm	0.0 a	0.0 b	0.0 a	0.0 a	0.0 b	0.0 b

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Grand total (GT) is the sum of the five monthly average total number of RFB forms recorded for each treatment.

the wheat, as suggested by data from bioassays, and by other workers (LaHUE and KADOUM, 1979). As a result, no larvae were recovered from malathion treated wheat and all adults recovered were dead. The greater recovery of adults from malathion when compared to other treatments can also be explained by the same rationale as for LGB adults.

Total recovery of adults from control treatments was very close to the expected value of 2.2 adults/5 kg for no increase in adult population. Thus, data suggested that RFB adults did not successfully reproduce in control wheat used in this experiment. This is in agreement with the belief that the RFB is not a primary pest of unmilled products (USDA, 1980), and that its rate of reproduction in grain is much slower than in flour (COTTON and WILBUR, 1974), particularly in wheat with little damage or dockage (McGREGOR, 1964). Wheat used in this experiment contained a low percentage of dockage.

Evaluation of protectant effectiveness was not possible because the RFB did not reproduce successfully in control wheat. However, it was clear that malathion was effective in killing adults and avoiding production of F-1 individuals, since all adults collected were dead and no larvae were found in malathion treatments. Methoprene also was effective according to its particular mode of action: it apparently prevented RFB F-1 larvae from transforming into adults. This is also supported by the bioassay experiments conducted during this study. Estimation of the actual damage and contamination by these long-living larvae would seem to be a better means of assessing methoprene as a grain protectant. Further investigation of this topic is

recommended.

Sawtoothed grain beetle. The grand total (GT) numbers of all STGB forms in the contol bins were significantly higher than those for malathion or 20-ppm methoprene, as shown in Table 9. The 10-ppm methoprene and control treatments were not significantly different nor could significance be distinguished between 10-ppm methoprene, 20-ppm methoprene or malathion treatments.

Malathion showed the lowest GT number of all STGB forms recovered (3.2 insects/5 kg); this recovery was close to the expected value of 2.2 insects/5 kg, for no STGB population increase. Table 9 indicates that all forms recovered from the malathion-treated wheat were adults, and no larvae (live or dead) were recovered. The same applies for the 20-ppm methoprene treatments. A few larvae were found in the 10-ppm methoprene treatments. The GT numbers of larvae recovered from the control bins were significantly different fromthe GT number of larvae collected from any of the other treatments indicating that the STGB was actively reproducing in untreated wheat. No significant differences were found in the numbers of larvae in methoprene or malathion treatments.

Significant differences were found in the first four monthly samplings between the number of larvae recovered from the controls and the other treatments.

No significant differences were found in the number of adults recovered from any of the treatments, at any monthly sampling or when comparing the GT number of adults (Table 9). However, the GT number of adults in control wheat was

Table 9. Average total numbers of live and dead sawtoothed grain beetle (per 1,000 g) from 3,000-lb lots of wheat at monthly intervals after treatment, during 5-months storage.[ab]

TREATMENT	MONTHS AFTER TREATMENT					GRAND TOTAL[c]
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
			ALL FORMS			
CONTROL	3.6 a	9.7 a	7.5 a	3.1 a	10.3 a	34.2 a
METHOPRENE 10 ppm	0.0 b	3.0 a	4.7 ab	2.5 a	2.5 b	12.6 ab
METHPORENE 20 ppm	0.4 b	2.2 a	1.5 b	1.4 a	1.5 b	6.9 b
MALATHION 10 ppm	0.4 b	1.4 a	0.4 b	0.7 a	0.4 b	3.2 b
			ADULTS			
CONTROL	1.4 a	7.9 a	4.3 a	2.4 a	7.4 a	23.4 a
METHOPRENE 10 ppm	0.0 a	3.0 a	3.6 a	2.5 a	2.5 a	11.5 a
METHOPRENE 20 ppm	0.4 a	2.2 a	1.5 a	1.4 a	1.5 a	6.9 a
MALATHION 10 ppm	0.4 a	1.4 a	0.4 a	0.7 a	0.4 a	3.2 a
			LARVAE			
CONTROL	2.2 a	1.8 a	3.2 a	0.7 a	2.9 a	10.8 a
METHOPRENE 10 ppm	0.0 b	0.0 b	1.1 b	0.0 b	0.0 a	1.1 b
METHOPRENE 20 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 a	0.0 b
MALATHION 10 ppm	0.0 b	0.0 b	0.0 b	0.0 b	0.0 a	0.0 b

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Grand total (GT) is the sum of the five monthly average total number of STG forms recorded for each treatment.

approximately twice the GT number of adults from 10-ppm methoprene; three times greater than the GT number of adults from 20-ppm methoprene; and seven times greater than the GT number of adults from 10-ppm malathion. Thus, the GT number of STGB adults in samples was not as good an indicator as the total number of all STGB forms (adults, larvae and pupae together), in detecting differences between treatments. The number of larvae was the best variable in detecting differences between treatments, either monthly or for the GTs. Additionally, the numbers of larvae in samples gave the best indication as to whether or not the population of STGB insects in a bin was reproducing successfully. MCGREGOR and KRAMER (1975), and STRONG and DIEKMAN (1973) reported that STGB adults exposed to methoprene-treated media were not killed. Therefore, live adults found in samples of methoprene-treated grain may not be reproducing successfully, as suggested by results obtained in this study. It is important to consider these facts when monitoring insect populations in grain that has been treated with methoprene, or products of a similar mode of action.

In general, levels of STGB insects found in samples from control bins were high, if compared with the other species in the same control samples, i.e., LGB, RFB, and FGB. In fact, no other beetle species tested in this experiment appeared as frequently as the STGB in the monthly control samples. The STGB was the most successful of all species tested. This species has been called the most important pest of farm-stored grain in England (MINISTRY of AGRICULTURE, FISHERIES AND FOOD, 1976). In Kansas, two similar surveys (unpublished) of farm-stored grain, one

conducted in 1965 and the other in 1971, showed that the STGB was one of the most predominant species infesting wheat. In the 1965 survey, the STGB was not only the species most frequently found, but it also showed the highest level of infestation per kilogram of sample.

The two levels of methoprene tested were found to be statistically equivalent throughout the experiment, with respect to the total number of insects, adults, or larvae of the STGB. All analyses indicated that there were no differences between methoprene treatments and the malathion treatment. Thus, protection of wheat against this species by the three treatments was statistically equivalent during the storage period.

Flat grain beetle. The three grand total (GT) numbers of all forms, adults, and larvae were significantly greater in the controls than in the other treatments (Table 10). No significant differences were found between methoprene or malathion treatments, in numbers of all forms, adults, or larvae recovered in the monthly samplings or for the GTs. Data clearly indicate that the FGB was able to reproduce successfully only in the control treatments. Malathion and 20-ppm methoprene treatments did not yield any FGB larvae throughout the experiment, and provided an excellent protection of the wheat. The 10-ppm methoprene treatment showed a GT of just 1.1 larvae/5 kg, also indicating effective protection. The point of complete inhibition lies somewhere between 10- and 20- ppm methoprene, according to results obtained in this experiment.

With few exceptions, monthly samplings also showed that total numbers of all forms, adults, or larvae found in control

Table 10. Average total numbers of live and dead flat grain beetle forms (per 1,000 g) from 3,000-lb lots of wheat at monthly intervals after treatment, during 5-months storage.[ab]

TREATMENT	MONTHS AFTER TREATMENT					GRAND TOTAL[c]
	1 (SEP)	2 (OCT)	3 (NOV)	4 (DEC)	5 (JAN)	
			ALL FORMS			
CONTROL	2.5 a	7.3 a	6.1 a	3.1 a	7.1 a	26.1 a
METHOPRENE 10 ppm	0.0 b	1.1 b	0.4 b	0.4 b	0.0 b	1.8 b
METHOPRENE 20 ppm	0.4 b	1.1 b	0.0 b	0.0 b	0.0 b	1.4 b
MALATHION 10 ppm	1.4 b	0.0 b	0.0 b	0.0 b	0.0 b	1.4 b
			ADULTS			
CONTROL	1.8 a	6.6 a	4.3 a	1.0 a	2.8 a	16.5 a
METHOPRENE 10 ppm	0.0 a	0.4 b	0.4 b	0.0 b	0.0 b	0.7 b
METHOPRENE 20 ppm	0.4 a	1.1 b	0.0 b	0.0 b	0.0 b	1.4 b
MALATHION 10 ppm	1.4 a	0.0 b	0.0 b	0.0 b	0.0 b	1.4 b
			LARVAE			
CONTROL	0.7 a	0.7 a	1.8 a	2.1 a	4.3 a	9.6 a
METHOPRENE 10 ppm	0.0 b	0.7 a	0.0 b	0.4 ab	0.0 b	1.1 b
METHOPRENE 20 ppm	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b
MALATHION 10 ppm	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Grand total (GT) is the sum of the five monthly average total number of FGB forms recorded for each treatment.

bins were significantly higher than the numbers obtained from treated bins. Thus, monthly samplings also showed that methoprene and malathion treatments were equally effective in controlling FGB.

Previous literature reporting methoprene as a grain protectant did not mention the FGB; therefore comparisons with published data were not possible.

BIOASSAYS.

Survival and development of progeny.

Parent adult mortality.

Mortalities of parent adults after an exposure period of seven days to treated wheat are reported in Table 11. All four species tested (LGB, RFB, STGB, and FGB) showed high mortality only when exposed to malathion-treated wheat. This supports the assumption about the clumped distribution of insects in malathion-treated wheat. This assumption was used to explain the occasional high insect recoveries from malathion-treated wheat during monthly sampings.

Bioassays 1, 2, and 3 (7, 45 and 90 days after treatment, respectively) indicated that methoprene treatments were not lethal to LGB or RFB parent adults. STGB and FGB species did show some mortality differences between methoprene and control treatments. These differences, however, were not consistent and were small.

In this experiment, exposure of insects to treated wheat occurred under "ventilated" conditions, i.e.,lids used in bioassay jars did not limit gaseous exchange with surrounding

Table 11. Beetle adult mortalities after 7 days in 50-g lots of wheat when bioassayed with 25 adults at 7, 45, and 90 days after treatment.[a]

TREATMENT	DAYS AFTER TREATMENT		
	7	45	90
	LESSER GRAIN BORER		
CONTROL	0.44 b	1.33 b	0.44 b
METHOPRENE 10 ppm	0.00 b	0.00 b	0.44 b
METHOPRENE 20 ppm	0.00 b	0.00 b	1.35 b
MALATHION 10 ppm	99.56 a	96.00 a	89.70 a
	RED FLOUR BEETLE		
CONTROL	0.89 b	0.00 b	0.00 b
METHOPRENE 10 ppm	1.33 b	0.44 b	0.00 b
METHOPRENE 20 ppm	1.78 b	0.89 b	0.00 b
MALATHION 10 ppm	100.00 a	100.00 a	99.09 a
	SAWTOOTHED GRAIN BEETLE		
CONTROL	0.00 c	1.33 b	0.44 b
METHOPRENE 10 ppm	0.44 c	2.22 b	1.33 b
METHOPRENE 20 ppm	4.00 b	1.56 b	1.78 b
MALATHION 10 ppm	100.00 a	100.00 a	100.00 a
	FLAT GRAIN BEETLE		
CONTROL	0.89 c	0.89 b	0.00 c
METHOPRENE 10 ppm	2.22 bc	1.33 b	3.11 bc
METHOPRENE 20 ppm	4.02 b	2.22 b	4.44 b
MALATHION 10 ppm	100.00 a	100.00 a	100.00 a

[a]Average of three replications' means. Each mean was the average of three repeated measures.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

atmosphere. Considering this, parent adult mortality results are in agreement with those reported by STRONG and DIEKMAN (1973), and MCGREGOR and KRAMER (1975).

Progeny assessment.

Progenies of the four species were assessed by counting adults of the LGB, and adults, larvae and pupae of the other three beetle species in 50-g bioassay samples after 50 days.

Lesser grain borer. Numbers of LGB progeny in control samples were statistically greater than those in the protectant-treated samples, in all three bioassays (Table 12). With the exception of one adult found in 20-ppm methoprene in the first bioassay, there were no LGB progeny in protectant-treated samples. The other two bioassays confirm that methoprene at 20 ppm was effective in controlling LGB progeny.

Methoprene treatments did not kill LGB adults during the seven-day exposure (Table 11). During this period LGB adults were expected to lay eggs. However, X-ray results at 80 days (Tables 22 and 23) showed that methoprene-treated samples were not invaded by immature forms of the LGB species. MIAN and MULLA (1982b) reported that oviposition of LGB adults was not affected by methoprene at 5 ppm in wheat, but that the ovicidal activity of the chemical was what effectively controlled the progeny (100% control).

Methoprene may be considered an ideal JH compound for the control of LGB, since it apparently inhibits the development of the egg stage, thus avoiding the appearance of the larvae which can cause greater food losses (WILLIAMS and AMOS, 1974).

Table 12. Lesser grain borer progeny after 50 days in 50-g lots of wheat when bioassayed with 25 adults for 7, 45 and 90 days after treatment.[ab]

TREATMENT	DAYS AFTER TREATMENT		
	7	45	90
	ADULTS		
CONTROL	65.00 a	113.78 a	11.89 a
METHOPRENE 10 ppm	0.00 b	0.00 b	0.00 b
METHOPRENE 20 ppm	0.11 b	0.00 b	0.00 b
MALATHION 10 ppm	0.00 b	0.00 b	0.00 b

[a]Adults originally placed in jars containing the treated wheat were removed after 7 days.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

Red flour beetle. Few adult RFB progeny were found in control samples (Table 13). This agrees with results obtained during monthly samplings of the bin experiment, in which RFB showed no increase in population.

Pupae were produced only in control samples; none in malathion or methoprene treatments. The malathion treatment also gave complete control of RFB larvae. This may be associated with the high parent adult mortality in the malathion-treated samples (Table 11). The RFB adults (10%) which survived the seven-day exposure period to malathion during the third bioassay, were apparently not able to produce progeny.

One adult was found in malathion samples during the second bioassay. Apart from this, no other evidence of RFB progeny was observed in any of the three bioassays.

Methoprene treatments permitted the development of RFB larvae, although generally fewer than in the controls (statistically different in first and second bioassays). No evidence was found during the bioassays of RFB larvae attempting to pupate. WILLIAMS and AMOS (1974) also reported complete suppression of RFB progeny in wheat flour at 5- and 20- ppm methoprene.

Sawtoothed grain beetle. With one exception, STGB progeny occurred only in control samples in all three bioassays (Table 14) (larvae were found in the 10-ppm methoprene samples during the second bioassay). This agrees with results obtained in the bin experiment. Particularly, the near complete absence of larvae in bioassays confirms findings in the bin experiment. Both the bioassays and the bin experiment indicated that

Table 13. Red flour beetle progeny after 50 days in 50-g lots of wheat when bioassayed with 25 adults for 7 days at 7, 45 and 90 days after treatment.[a]

TREATMENT	DAYS AFTER TREATMENT								
	7			45			90		
	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE
CONTROL	0.9 a	19.9 a	13.2 a	0.7 a	14.7 a	26.2 a	0.0 a	70.4 a	22.2 a
METHOPRENE 10 ppm	0.0 b	7.3 bc	0.0 b	0.0 b	9.9 a	0.0 b	0.0 b	24.9 b	0.0 b
METHOPRENE 20 ppm	0.0 b	8.4 b	0.0 b	0.0 b	6.2 ab	0.0 b	0.0 c	16.0 b	0.0 b
MALATHION 10 ppm	0.0 b	0.0 c	0.0 b	0.4 ab	0.0 b	0.0 b	0.0 d	0.0 c	0.0 b

[a]Numbers followed by the same letter in the same column are no significantly different at the 5% level using LSD test.

Table 14. Sawtoothed grain beetle progeny after 50 days in 50-g lots of wheat when bioassayed with 25 adults for 7 days at 7, 45 and 90 days after treatment.[a]

TREATMENT	DAYS AFTER TREATMENT								
	7			45			90		
	ADULT	LARVAE	PUPAE	ADULT	LARVAE	PUPAE	ADULT	LARVAE	PUPAE
CONTROL	42.3 a	1.0 a	6.0 a	40.4 a	5.2 a	16.8 a	80.4 a	4.0 a	0.6 a
METHOPRENE 10 ppm	0.0 b	0.0 a	0.0 b	0.0 b	0.7 b	0.0 b	0.0 b	0.0 b	0.0 b
METHOPRENE 20 ppm	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
MALATHION 10 ppm	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b

[a]Numbers followed by the same letter in the same column are not significantly different at 5% level using LSD test.

methoprene was effective against the STGB. The inhibitory effect probably occurred at an early stage during development. MIAN and MULLA (1982b) indicated that control of the STGB by methoprene occurs mainly as result of high mortality of eggs and first instar larvae.

Flat grain beetle. Progenies of FGB in control samples were significantly greater than in any of the treated samples (Table 15), and no statistical differences were found among the protectant treatments.

Pupae were not detected in protectant-treated samples; a few adults were found only during the second bioassay; and a few larvae were recorded in the 10-ppm methoprene treatments in the second and third bioassays. Protectants effectively controlled progeny of the FGB. This agrees with results from the bin experiment, where FGB populations increased only in control wheat.

Indian meal moth. In all three bioassays, numbers of adult progeny in control samples were significantly greater than that in protectant-treated samples (Table 16). No IMM adults were produced in methoprene-treated samples, while several were produced in malathion-treated samples in all three bioassays; but differences between methoprene and malathion treatments were significant only in the second bioassay. Bioassay data showed that some IMM eggs survived the exposure to malathion-treated wheat, and were able to reach adulthood, although in numbers significantly lower than in control treatments.

Bin experiment results indicated that a few IMM adults were recovered from methoprene-treated wheat over the 5-month storage

Table 15. Flat grain beetle progeny after 50 days in 50-g lots of wheat when bioassayed with 25 adults for 7 days at 7, 45 and 90 days after treatment.[a]

TREATMENT	DAYS AFTER TREATMENT 7			45			90		
	ADULT	LARVAE	PUPAE	ADULT	LARVAE	PUPAE	ADULT	LARVAE	PUPAE
CONTROL	15.7 a	2.6 a	9.8 a	3.0 a	10.2 a	3.6 a	0.4 a	26.1 a	11.4 a
METHOPRENE 10 ppm	0.0 b	0.0 b	0.0 b	0.2 b	0.3 b	0.0 b	0.0 b	0.4 b	0.0 b
METHOPRENE 20 ppm	0.0 b	0.0 b	0.0 b	0.1 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
MALATHION 10 ppm	0.0 b	0.0 b	0.0 b	0.1 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b

[a]Numbers followed by the same letter in the same column are no significantly different at the 5% level using LSD test.

Table 16. Indian meal moth progeny after 50 days in 50-g lots wheat when bioassayed with 25 eggs at 7, 45 and 90 days after treatment.[a]

TREATMENT	DAYS AFTER TREATMENT								
	7			45			90		
	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE
CONTROL	6.2 a	1.6 b	0.7 a	9.3 a	28.3 a	0.0 b	10.9 a	46.6 a	0.0 b
METHOPRENE 10 ppm	0.0 b	5.2 a	0.0 a	0.0 c	3.7 b	0.0 b	0.0 b	4.1 b	0.0 b
METHOPRENE 20 ppm	0.0 b	4.8 a	0.0 a	0.0 c	4.4 b	0.0 b	0.0 b	4.2 b	0.0 b
MALATHION 10 ppm	1.4 b	0.0 c	0.7 a	3.3 b	8.7 b	0.6 a	1.2 b	3.6 b	1.1 a

[a]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

period studied. This contrasts with bioassay data, however. Bioassay results are in agreement with those reported by MCGREGOR and KRAMER (1975), who found that wheat treated with methoprene at 2 ppm prevented development of the immature larvae of the Indian meal moth.

IMM progenies in methoprene-treated samples were limited to larvae. No pupae or adults were produced. No evidence was observed of successful metamorphosis.

Assessment of population development.

The ability of five insect species to establish populations in treated wheat was assessed in jars containing 100 g of infested wheat by counting adults, larvae, and pupae at the end of a 100-day period.

Lesser grain borer. Data showed that LGB established populations only in control samples (Table 17). A few LGB adults were recovered from malathion-treated samples, however, the presence of those few insects cannot be considered an indication of a successful LGB population over the period studied (100 days). X-ray data presented in Tables 22 and 23 indicated that this species was not successful in reproducing in malathion-treated samples. Both methoprene treatments were also effective in preventing LGB from establishing populations.

Red flour beetle. RFB's established populations only in control samples (Table 18). No insect forms were found in malathion-treated samples during the three bioassays. Only larvae which were not successful in completing metamorphosis after 100 days were recovered from methoprene-treated samples. Some of these

Table 17. Lesser grain borer adult populations after 100 days in 100-g lots of wheat when bioassayed with 50 adults at 90 days after treatment.[ab]

TREATMENT	THIRD BIOASSAY (90 days after treatment)
	ADULTS
CONTROL	1482.0 a
METHOPRENE 10 ppm	0.0 b
METHOPRENE 20 ppm	0.0 b
MALATHION 10 ppm	1.7 b

[a]Parent adults (50) not included in calculations.
[B]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

larvae were still alive, were larger in size and had a more heavily sclerotized integument than normal larvae from control samples. LOSCHIAVO (1976) reported RFB larvae which survived 120 days or longer and which also exhibited physical characteristics similar to those observed in this experiment.

No evidence was found that larvae developing in methoprene-treated wheat attempted to undergo pupation, nor were any other intermediate stages observed. WILLIAMS and AMOS (1974) reported the emergence of RFB adultoids when insects were reared in flour milled from wheat treated with methoprene at 5 and 20 ppm. It is possible that the medium used in this experiment (whole wheat kernels) imposed limitations to complete metamorphosis on the RFB insects, additional to those imposed by the protectant.

Sawtoothed grain beetle. Data indicated that STGB established a population only in control samples (Table 19). Methoprene and malathion treatments completely prevented the appearance of progeny. These results agreed with progeny assessment results (Table 27) and results of the bin experiment (Table 9).

Flat grain beetle. FGB insects established a population only in control samples (Table 20). All protectant treatments completely prevented the appearance of progeny, and thus the establishment of a population. These results support those reported in Tables 15 (progeny assessment), and those in Table 10 (bin experiment).

Indian meal moth. IMM established populations in control and in malathion samples (Table 21), however, adult populations in control samples were significantly larger than in malathion

Table 18. Red flour beetle populations after 100 days in 100-g lots of wheat when bioassayed with 50 adults at 7, 45 and 90 days.[ab]

TREATMENT	DAYS AFTER TREATMENT 7			45			90		
	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE
CONTROL	7.0 a	46.0 a	14.0 a	64.7 a	91.3 a	10.3 a	50.0 a	152.7 a	4.3 a
METHOPRENE 10 ppm	0.0 b	32.0 b	0.0 b	0.0 b	80.3 a	0.0 b	0.0 b	106.3 b	0.0 b
METHOPRENE 20 ppm	0.0 b	17.3 c	0.0 b	0.0 b	34.0 b	0.0 b	0.0 b	51.3 c	0.0 b
MALATHION 10 ppm	0.0 b	0.0 d	0.0 b	0.0 b	0.0 b[c]	0.0 b	0.0 b	0.0 d	0.0 b

[a]Parent adults (50) not inclyded in calculations.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.
[c]Least significant difference = 43.25.

Table 19. Sawtoothed grain beetle populations after 100 days in 100-g lots of wheat when bioassayed with 50 adults at 7, 45 and 90 days after treatment.[ab]

TREATMENT	THIRD BIOASSAY (90 days after treatment)		
	ADULTS	LARVAE	PUPAE
CONTROL	96.0 a	7.7 a	0.0 a
METHOPRENE 10 ppm	0.0 b	0.0 b	0.0 b
METHOPRENE 20 ppm	0.0 b	0.0 b	0.0 c
MALATHION 10 ppm	0.0 b	0.0 b	0.0 d

[a]Parent adults (50) not included in calculations.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

Table 20. Flat grain beetle populations after 100 days in 100-g lots of wheat when bioassayed with 50 adults at 7, 45 and 90 days after treatment.[ab]

TREATMENT	THIRD BIOASSAY (90 days after treatment)		
	ADULTS	LARVAE	PUPAE
CONTROL	291.3 a	5.3 a	0.3 a
METHOPRENE 10 ppm	0.0 b	0.0 b	0.0 a
METHOPRENE 20 ppm	0.0 b	0.0 b	0.0 a
MALATHION 10 ppm	0.0 b	0.0 b	0.0 a

[a]Parent adults (50) not included in calculations.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

Table 21. Indian meal moth populations after 100 days in 100-g lots of wheat when bioassayed with 50 eggs at 7, 45 and 90 days after treatment.[ab]

TREATMENT	DAYS AFTER TREATMENT 7			45			90		
	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE	ADULTS	LARVAE	PUPAE
CONTROL	12.7 a	14.7 a	1.3 a	43.3 a	23.3 a	1.0 ab	30.7 a	11.7 b	1.7 a
METHOPRENE 10 ppm	0.0 c	6.7 a	0.0 b	0.0 c	11.0 a	0.0 b	0.0 c	5.7 c	0.0 b
METHOPRENE 20 ppm	0.0 c	7.7 a	0.0 b	0.0 c	9.7 a	0.0 b	0.0 c	8.3 bc	0.0 b
MALATHION 10 ppm	5.0 b	12.3 a	0.3 ab	19.0 b	37.7 a	3.3 a	22.0 b	25.0 a	0.7 ab

[a]Parent adults (50) not included in calculations.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

bioassays. This indicated that malathion treatment, posed some limitation on population development; possibly through elimination of less resistant individuals.

Bin experiment results suggested that IMM populations were increasing in malathion-treated bins by the third month. Bioassay results confirmed the capability of the IMM laboratory strain used in this experiment to overcome the malathion treatment. Resistance to malathion in IMM populations is widespread as reported by BANSODE et al (1981), LaHUE (1969), and ZETTLER et al (1973).

On the other hand, methoprene treatments allowed only the presence of larvae and a population was not established. This agrees with results on progeny assessment (Table 16), and also with results from the bin experiment (Table 4). In the latter, some adult insects were reported in the methoprene-treated bins, however, no adults were found in either the bioassay for progeny assessment or in the bioassay for population development assessment.

Internal infestation by lesser grain borer. All bioassay samples infested with LGB adults (100-g amd 50-g samples) were X-rayed after 80 days.

X-rays clearly indicated that LGB insects were highly successful in control samples (Tables 22 and 23). This did not agree with results obtained in the bin experiment, where LGB insects were apparently not able to increase their numbers in untreated wheat.

The analysis of internal infestation in protectant-treated samples indicated effective protection against immature LGB

Table 22. Lesser grain borer internal infestation after 80 days in 50-g lots of wheat bioassayed with 25 adults at 7, 45 and 90 days after treatment.[abcd]

TREATMENTS	DAYS AFTER TREATMENT		
	7	45	90
CONTROL	110.22 a	330.67 a	21.22
METHOPRENE 10 ppm	0.78 b	0.44 b	0.78
METHOPRENE 20 ppm	0.67 b	0.44 b	0.56
MALATHION 10 ppm	0.00 b	0.00 b	0.00

[a]Average of three replications.
[b]Data indicates sum of all adults, larvae, and pupae detected in kernels by X-ray photography.
[c]Methoprene treatments showed only larvae.
[d]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

Table 23. Lesser grain borer internal infestation after 80 days in 100-g lots of wheat bioassayed with 50 adults at 7, 45 and 90 days after treatment.[abcd]

TREATMENT	DAYS AFTER TREATMENT		
	7	45	90
CONTROL	776.67 a	866.67 a	712.00 a
METHOPRENE 10 ppm	1.00 b	1.33 b	1.33 b
METHOPRENE 20 ppm	0.00 b	1.00 b	3.00 b
MALATHION 10 ppm	0.33 b	0.00 b	1.00 b

[a]Average of three replications.
[b]Data indicates sum of all adults, larvae and pupae detected in kernels by X-ray photography.
[c]Methoprene treatments showed only larvae.
[d]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

insect invasion through the third bioassay (90 days after treatments). Thus, the LGB adults which survived the malathion or methoprene treatments during the third bioassays (Table 11), were not effectively reproducing.

Percent degermed kernels. All IMM bioassays were analyzed for degermed kernels after 50 and 100 days. In general, determinations made at 50 days in either type of bioassay (50- or 100-g) showed malathion-treated samples with the lowest percent of degermed kernels (Tables 24 and 25). The same was observed at 100 days when the 50-g jars were analyzed, however, after 100 days the 100-g malathion-treated samples showed a substantial increase in the percent of degermed kernels. In the second bioassay, damage in malathion samples was not statistically different from the other treatments. In the third bioassay, damage was the greatest, and not significantly different from that of the control samples. At 50 days methoprene-treated samples showed the highest or next to highest percent of degermed kernels, however, at 100 days these samples were usually surpassed by control samples, although the differences were not always significant.

IMM insects were able to produce an F-1 generation in both control and malathion-treated samples, but not in methoprene-treated samples (Tables 16, and 21). This explains why, after 100 days, control and malathion-treated samples had higher levels of degermed kernels. After 100 days, protection of wheat by methoprene against germ damage by IMM insects was better than malathion.

Table 24. Percent kernels degermed by IMM after 50 and 100 days in 50-g lots of wheat bioassayed with 25 eggs.[ab]

TREATMENT	DAYS AFTER TREATMENT					
	7		45		90	
	DAYS AFTER INFESTATION					
	50	100	50	100	50	100
CONTROL	31.0 a	34.7 b	35.2 b	60.3 a	46.7 a	78.2 a
METHOPRENE 10 ppm	30.4 a	55.9 a	45.7 a	53.0 a	43.2 a	52.7 b
METHOPRENE 20 ppm	22.1 b	34.4 b	47.7 a	57.9 a	39.1 a	45.0 b
MALATHION 10 ppm	6.3 c	10.0 c	10.3 c	33.3 b	14.2 b	18.7 c

[a]Average of three replications.
[b]Numbers followed by the samer letter in the same column are not significantly different at the 5% level using LSD test.

Table 25. Percent kernels degermed by IMM after 50 and 100 days in 100-g lots of wheat bioassayed with 50 eggs.[ab]

TREATMENT	DAYS AFTER TREATMENT					
	7		45		90	
	DAYS AFTER INFESTATION					
	50	100	50	100	50	100
CONTROL	22.0 b	54.7 a	40.0 c	72.7 a	43.0 ab	72.3 a
METHOPRENE 10 ppm	42.7 a	46.0 a	58.7 a	60.7 a	55.7 a	52.0 b
METHOPRENE 20 ppm	26.7 b	27.0 b	51.0 b	56.0 a	37.0 b	41.7 b
MALATHION 10 ppm	12.3 c	18.0 b	9.0 d	45.7 a	27.0 b	74.3 a

[a]Average of three replications.
[b]Numbers followed by the same letter in the same column are not significantly different at the 5% level using LSD test.

The 20-ppm methoprene treatment usually yielded fewer degermed kernels than the 10-ppm methoprene treatment, although differences were not always significant. No clear advantage was detected statistically for the use of methoprene at 20 ppm over the 10-ppm treatment, as far as protection of wheat kernels against IMM germ damage is concern.

SUMMARY AND CONCLUSIONS

Methoprene at two levels (10 and 20 ppm) was compared to malathion (10 ppm) as wheat protectant treatment against five species of stored-product insects (IMM, LGB, RFB, STGB, and FGB). Wheat in lots of 50 bu each were treated and placed in corrugated metal bins located in a pole building. Bins were infested at the beginning of the test, and thereafter insect populations were monitored by monthly samplings. Samples for bioassay studies were periodically obtained from bins.

All protectant treatments significantly reduced the total mumber of IMM forms recorded during five consecutive monthly bin inspections. Methoprene treatments had significantly smaller numbers of IMM than 10-ppm malathion. No significant differences were found between levels of methoprene (10 and 20 ppm).

The total of all LGB, RFB, STGB, and FGB forms (live and dead) recovered in 5 monthly samples, indicated that all three protectant treatments were statistically equivalent in protecting wheat, and infestation was significantly lower than in controls. Only dead adults were found in samples obtained from wheat lots treated with malathion. Both larvae and adults (live and dead) were found in methoprene-treated lots and in controls.

STGB was the beetle species that developed best in control wheat, followed by FGB, RFB, and LGB. LGB did not successfully increase its population in control bins. However, 250-g samples taken monthly and incubated for 50 days, showed that after 1 month wheat had been infested internally by LGB.

In bioassay tests, parent adults of all beetle species died in malathion-treated wheat. These tests showed that methoprene almost completely prevented F-1 progeny of LGB, STGB and FGB, when adult insects were exposed to treated wheat for 7 days. This agreed with observations made in the bin experiment. RFB and IMM species were able to produce larvae which lived longer than normal and were larger in size but apparently were not able to complete metamorphosis. This also agrees with data from bin experiment.

Bioassay tests indicated that beetle species were not able to establish populations in methoprene-treated wheat at either level, while IMM established populations in only malathion-treated wheat and in controls. This, again, support findings in the bin experiment.

After 50 days in methoprene-treated wheat, IMM larvae had degermed more kernels than larvae in control or malathion treatments. After 100 days the opposite was true.

Finally, it was concluded that, under the conditions of this experiment, protection by malathion or methoprene against the beetle species studied was equally effective, however, methoprene gave better protection against IMM.

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APPENDIX 1

Procedure to Calculate Protectant Dosages.

I. Calculation of ppm.

a. Enough solution, containing the protectant to be applied, for three replications (three bins) was prepared and placed in sprayer tank. Initial volume was measured, as well as volume remaining in tank after application; volume of solution used was recorded.

b. Time sprayer was delivering solution was marked down for each bin.

c. Total volume of solution used refers to solution applied to three consecutive bins. Amount of solution applied to each bin was found by dividing:

total solution used ÷ total spraying time = delivery rate

Then:

delivery rate x partial spraying time = solution applied to a 3,000 lb lot

d. solution applied to a bin load x concentration of protectant in solution = ml protectant applied

Then: ml of protectant applied x g protectant/ml formulation = g of protectant applied

Then: g of protectant ÷ 1361 Kg/lot of wheat = ppm

II. Summary of data used in calculations.

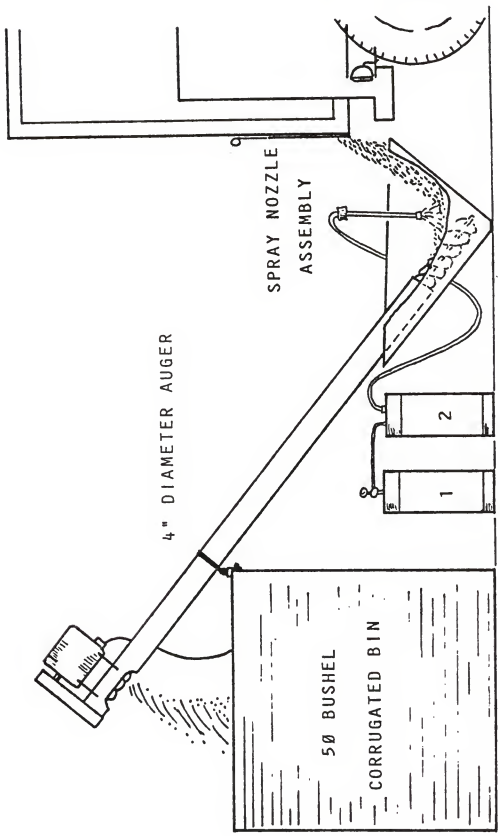
TREATMENT	DELIVERY RATE (ml/min)	PROTECTANT CONC IN SOL (ml/l)	SPRAYING TIME (min)	PROTECTANT CONCENTRATION IN WHEAT (ppm)
CONTROL				
Bin 1	132.9	N/A	13:33	N/A
Bin 2	132.9	N/A	14:30	N/A
Bin 3	132.9	N/A	14:23	N/A

(continue)
II. Summary of data used in calculations.

TREATMENT	DELIVERY RATE	PROTECTANT CONCENTRATION IN SOLUTION	SPRAYING TIME (min)	PROTECTANT CONCENTRATION IN TREATED WHEAT
METHOPRENE (10ppm)				
Bin 4	132.7	12.06	13:30	9.53
Bin 5	132.7	12.06	14:30	10.23
Bin 6	132.7	12.06	14:00	9.88
METHOPRENE (20ppm)				
Bin 7	126.8	24.12	14:45	19.88
Bin 8	126.8	24.12	15:45	21.24
Bin 9	126.8	24.12	15:00	20.22
MALATHION (10ppm)				
Bin 10	125.1	12.58	14:40	9.67
Bin 11	125.1	12.58	14:50	9.78
Bin 12	125.1	12.58	15:10	10.00

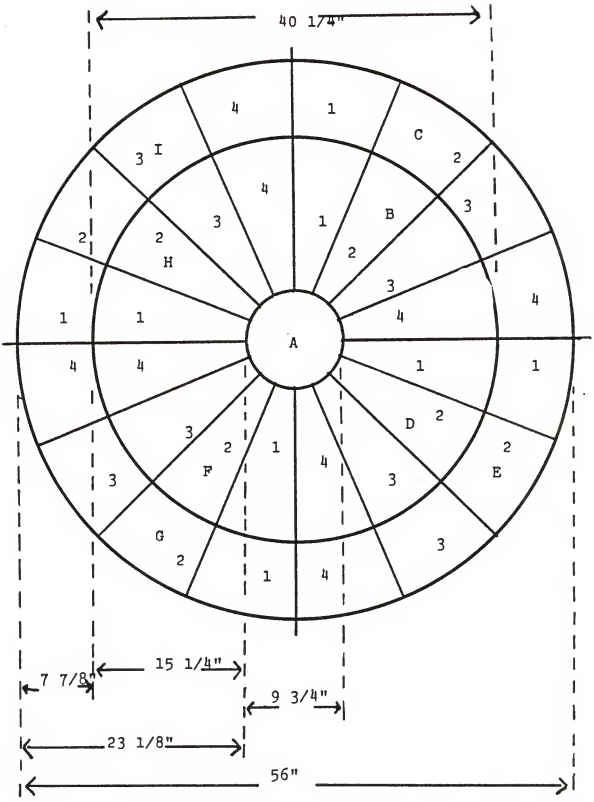
Appendix 2

Insecticide Application System



Appendix 3

Template for Bin Sampling



APPENDIX 4

Analytical procedure for malathion residues in grain.

The outline below indicates the standard analytical procedure used in the U.S. Grain Marketing Research Laboratory, Manhattan, Kansas, for determination of malathion residues in grain.

I. Extraction. Wheat (approx. 100 g) is ground twice in a Hobart coffee grinder (The Hobart Mfg Co., Troy, Ohio). A 25-g portion of the ground material was placed in a Sorvall Omnimixer (Ivan Sorvall Inc., Newton, Connecticut) with 75 ml acetonitrile saturated with hexane and blended at maximum speed for 2 min. After a resting period of 2 min, the sample is blended again for 3 min, and allowed to settle for 10 min. The extract is decanted into a funnel fitted with qualitative filter paper and collected. Two 3-ml aliquotes are pipetted into a column packed with 2 g of adsorbent mixture (24 g Celite 545, 12 g MgO, and 15 g Novit SG-1 Charcoal). Just before use columns are rinsed successively with ethyl acetate, benzene and ethylacetate. The liquid sample is then pushed slowly through the column with N_2 until only 2-3 mm remained on top of the column. The sides of the column are washed with 2-3 ml of ethyl acetate. Then, the residue is eluted with 25 ml of ethyl acetatebenzene (25:75 v/v). Finally, solvents are pushed through the column with N_2. The elute is evaporated to dryness on a rotary evaporator at 30 C, the residue is dissolved in 6 ml hexane, transferred to a small graduated vial and frozen for analysis by gas-liquid chromatography. The most concentrated samples can be diluted with hexane before injection in the chromatograph.

II. Gas chromatography. A Tracor 560 gas chromatograph (Tracor Inc., Austin, Texas) equipped with a Nitrogen-Phosphorus detector was used to analyze for malathion. The pyrex glass column (1.2 m x 2mm I. D.) was packed with 3% DEGS on 100/120 mesh chromosorb WAW-DMCS obtained from Supelco Inc. (Supelco Inc., Bellefonte, Pennsylvania). The detector temperature was 250 C; oven temperature, 180 C; and inlet, 215 C. Helium carrier flow was 25 ml/min. The detector gas flows were 2.5 ml/min and 120 ml/min for hydrogen and air, respectively. Aliquots of 4 ul were injected with a microsyringe. Sample injections were alternated with injections of the 98% malathion standard solution (1, 2, 4, 6 and 8 ul of standard were used). An Omniscribe Stripchart Recorder (Houston Instruments, Austin, Texas) at a chart speed of 0.5 inch/min was used to record the output. Peak heights were measured. All determinations were repeated at least twice. A concentration vs peak height curve was plotted for the malathion standard, and the amount of malathion in the samples calculated.

APPENDIX 5

Average Monthly Temperature and Humidity, Manhattan, Ks., 1983.

Average monthly temperature and relative humidity in Manhattan, Ks, during August-January of 1982-83, as recorded in K.S.U. campus weather station.[abc]

AIR PROPERTY	MONTH OF THE YEAR					
	AUG	SET	OCT	NOV	DEC	JAN
TEMPERATURE (C)	23.6	20.7	15.4	6.0	3.2	-0.4
RELATIVE HUMIDITY (%)	84.7	69.6	61.2	53.4	40.3	29.5

[a]Experimental site is about 2 miles away from Weather Recording Station.
[b]Only last two weeks of August.
[c]Only first two weeks of January.

APPENDIX 6

Numbers of Beetle Species Forms by Bin Level.

Average total recoveries by level of insects of all beetle species during the 5-month storage period studied.[ab]

TREATMENT	LEVEL IN BIN[C]	INSECT STAGE OF DEVELOPMENT			
		ADULTS		LARVAE	
		LIVE	DEAD	LIVE	DEAD
CONTROL	UPPER	9.0	8.7	1.3	3.3
	BOTTOM	7.7	16.0	4.3	14.3
METHOPRENE 10ppm	UPPER	2.0	4.0	1.0	4.3
	BOTTOM	4.3	4.0	0.3	7.0
METHOPRENE 20ppm	UPPER	3.3	3.0	1.3	1.3
	BOTTOM	1.7	3.3	0.7	3.7
MALATHION 10ppm	UPPER	0.0	9.0	0.0	0.0
	BOTTOM	0.0	8.3	0.0	0.0

[a]Average of three replications.
[b]Beetle species were: LGB, RFB, STGB, and FGB.
[C]Upper=upper half of bin; bottom=bottom half of bin.

APPENDIX 7

Water Vapor Pressures for Air and Stored Wheat.

Grain and air water vapor pressures for wheat stored during the 1982-83 winter in Manhattan, Kansas.[ab]

MONTH	TEMPERATURE (C)	RELATIVE HUMIDITY (%)	MOISTURE CONTENT (%w.b.)	VAPOR PRESSURE OF AIR (psia)	VAPOR PRESSURE OF GRAIN (psia)
AUG-SET	20.7	69.6	13.3	0.244	0.261
SET-OCT	15.4	61.2	13.7	0.157	0.195
OCT-NOV	6.0	53.4	13.5	0.073	0.102
NOV-DEC	3.2	40.3	13.5	0.046	0.084
DEC-JAN	-0.4	29.5	13.5	0.026	0.065

[a]Weather data taken at the K.S.U. Weather Station, located about 2 miles from experimental site.
[b]Calculated by using the Chung-Pfost equilibrium moisture content equation (1967).

METHOPRENE AS A PROTECTANT AGAINST FIVE SPECIES OF STORED-PRODUCT INSECTS IN WHEAT

by

MANUEL E. ZELEDON
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Abstract

Methoprene (an Insect Growth Regulator) was evaluated under semi-field conditions as a candidate wheat protectant against five species of stored-product insects, and compared to malathion. Newly-harvested wheat in lots of 50 bu each was treated and stored in metal bins in an open-sided building for 5 months. Groups of three bins each received the following treatments: control; 10-ppm methoprene; 20-ppm methoprene; and 10-ppm malathion. Bins were infested with adults of four species of stored-product beetles and eggs of Indian meal moths. Insect populations in bins were monitored through monthly samplings, with additional periodic samples to obtain grain for use in bioassays. Each insect species was bioassayed independently 7, 45 and 90 days after treatment, to determine parent adult mortality, progeny, and population establishment. In field studies, all protectant treatments significantly reduced the total number of Indian meal moths (IMM); however methoprene treatments (10 ppm and 20 ppm) had significantly smaller numbers of IMM than the 10-ppm malathion. The total of all insect stages (live and dead) of the lesser grain borer (LGB), red flour beetle (RFB), sawtoothed grain beetle (STGB) and flat grain beetle (FGB) recovered in 5 monthly samples indicated that all three protectant treatments were statistically equivalent in protecting wheat, and infestations were significantly lower than in controls. Both field and bioassay studies showed that methoprene treatments provided almost complete inhibition of F-1 progeny of

LGB, STGB and FGB. RFB and IMM produced larvae which lived longer than normal, were larger, but apparently were not able to complete metamorphosis. Bioassays showed that IMM larvae, after 50 days in methoprene-treated wheat, had degermed more kernels than larvae in controls or malathion treatments. After 100 days the opposite was true. Protection by malathion or methoprene treatments was equally effective for the beetle species studied, however methoprene gave better protection than malathion against IMM.